

Morphology of Gametophytes and Young Sporophytes of *Sphaeropteris lepifera*

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Abstract.—*Sphaeropteris lepifera* is one of the largest tree ferns in Taiwan. On average, it produces 50.7 sporangia per sorus, and 64 spores per sporangium. Spore germination, after 2 years of storage at 4°C was over 95%. The pattern of spore germination was “Cyathea-type”, and the gametophytes exhibited mainly Drynaria-type development with occasional Adiantum-type development. Typical gametophytes were heart-shaped but had the potential to elongate and become elliptical. Multicellular hairs on the dorsal and ventral surfaces of the midrib cushion increased in size and changed shape with age. They were usually uniseriate when young, and became multiseriate with age. Gametophytes initiated antheridia about 1 month after spores were sown, and did not become hermaphrodites until 7 weeks later. During ontogeny, the gametangial sequence was from the male to hermaphroditic. Antheridia formed on the wings of the ventral and dorsal surfaces of gametophytes. The wall of each antheridium was composed of 5 cells. Archegonia appeared on the cushion of the ventral surface of gametophytes. Some gametophytes initiated clone-formation through vegetative regeneration. When sufficient water was provided, young sporophytes began to appear 12 weeks after spores were sown. The first fronds were midribless. The uniseriate, multicellular hairs on young sporophytes were similar to those on gametophytes.

Gametophyte morphology, including mature forms, spore germination, and types of early development, trichomes, and gametangia, have been used to characterize fern taxa (Stokey, 1918, 1930; Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973). These characteristics provide information relevant to fern phylogeny (e.g., Nayar and Kaur, 1971) and the ecology and reproductive biology of different species (Chiou and Farrar, 1997a, b; Chiou *et al.*, 1998; Masuyama, 1975a, b, 1979).

In the Cyatheaceae, spore germination is Cyathea-type and gametophyte development is Adiantum-type or nearly Drynaria-type (Nayar and Kaur, 1971). Gametophytes are long-lived (2–6 years) and tend to elongate slightly with age. The multicellular, bristle-like hairs that appear on gametophytes in this family indicate the Cyathaceae is phylogenetically close to the Loxsomaceae (Atkinson and Stokey, 1964). Antheridia and archegonia typically occur on the same gametophytes, with some vigorous gametophytes being strictly archegonial (Stokey, 1930). Apogamy was reported in some species in the Cyatheaceae

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TABLE 1. Spore sources, usage, culture medium and storage period.

Spores	Location	Observation	Medium	Stored (years)
Huang 25	Wulai, 370 m	sporangium and spore number	—	0
Huang 26	Wulai, 370 m	sporangium and spore number	—	0
Huang 27	Yamingshan, 250 m	sporangium and spore number	—	0
Huang 25	Wulai, 370 m	germination rate	agar	0
Chiou 14495	Wulai, 150 m	germination rate	agar	2
Chiou 14787	Hsinihsien, 200 m	germination rate	agar	1
Huang 25	Wulai, 370 m	gametophyte morphology	peat	0
Huang 26	Wulai, 370 m	gametophyte morphology	peat	0
Huang 27	Yamingshan, 250 m	gametophyte morphology	peat	0

(Stokey, 1918), but Walker (1966) reinvestigated two of these species and found them to be sexual diploids.

Sphaeropteris lepifera (Hook.) Tryon (Cyatheaceae) is the largest, and one of the most abundant, species in this family in Taiwan. Wang *et al.* (1977) described its sporophyte morphology, scales, stomata, epidermal cells, and the anatomy of the stipes and trunks. However, except for the gametangial sequence (Masuyama, 1975b), characteristics of the gametophyte of *S. lepifera* have not been described.

The number of spores per sporangium has also been used in the systematic classification of ferns (Sen, 1964; Gastony, 1974). Spore viability, gametangial sequence, and gametophyte growth habits are important components of fern reproductive biology (Chiou and Farrar, 1997a; Chiou *et al.*, 1998). Complex interactions among gametophytes in multispore cultures may affect the sequence of gametangial development in individual plants. Detailed analysis of sexual status, in relation to the age and size of gametophytes, is essential for determining the most probable breeding system employed by *S. lepifera* (Chiou and Farrar, 1997b; Chiou *et al.*, 1998). The present study explores spore production, spore viability after cold storage, gametangial sequence, morphology and growth habits of the gametophytes and the morphology of young sporophytes of *S. lepifera*.

MATERIALS AND METHODS

Spores were collected from sporophytes growing on mountain slopes in northern Taiwan (Table 1). Voucher specimens were deposited in the Herbarium of Taiwan Forestry Research Institute (TAIF). Spores were stored in the refrigerator at 4°C.

The number of sporangia per sorus (from 50 randomly sampled sori per plant) and the number of spores per sporangium (from 50 randomly sporangia per plant) were estimated from three sporophytes (Table 1).

Spores were sown on peat or agar medium. The viability of fresh spores and spores stored at 4°C for one or two years was assessed (Table 1). Spores were sterilized with 2.5% Clorox for 5 min, rinsed with sterilized water for 10 min,

TABLE 2. Mean germination (%) of fresh and stored (4°C) *S. lepifera* spores sown on agar medium.

Storage (years)	Days after sowing spores			
	5	7	9	11
0	0.0 (0)	81.2 (7.2)	96.7 (4.0)	99.0 (1.2)
1	35.8 (26.0)	97.9 (2.0)	98.7 (1.5)	98.9 (1.5)
2	1.3 (1.9)	29.7 (16.8)	84.0 (7.4)	95.4 (4.0)

¹ Standard error in parentheses.

and then sown on 1.1% agar-solidified medium containing Gantt and Arnotts' (1965) mineral nutrients and one drop of 1% FeCl₃ per 400 ml of medium. The pH of the media ranged from 5.0–5.2. Germination rates were estimated from six agar-medium petri dish cultures with 50 spores per dish. Rupture of the spore wall was used as an indicator of germination. Gametophyte morphology was observed for 8 months. The sexual expression, age and size of 50 gametophytes from each of 3 multispore peat cultures were recorded every 2 weeks. Gametophyte width was measured at the widest point.

Because the peat medium provided conditions more similar to those in the field than the agar medium, gametophyte morphology and sexual expression were assessed only for gametophytes in peat-grown cultures. However, gametophyte cultures on agar medium were used to estimate germination because spores and their germination status could be seen more easily on agar medium. All cultures were maintained at 20–25°C under white fluorescent illumination of 1000–1500 lux for 12 hrs per day.

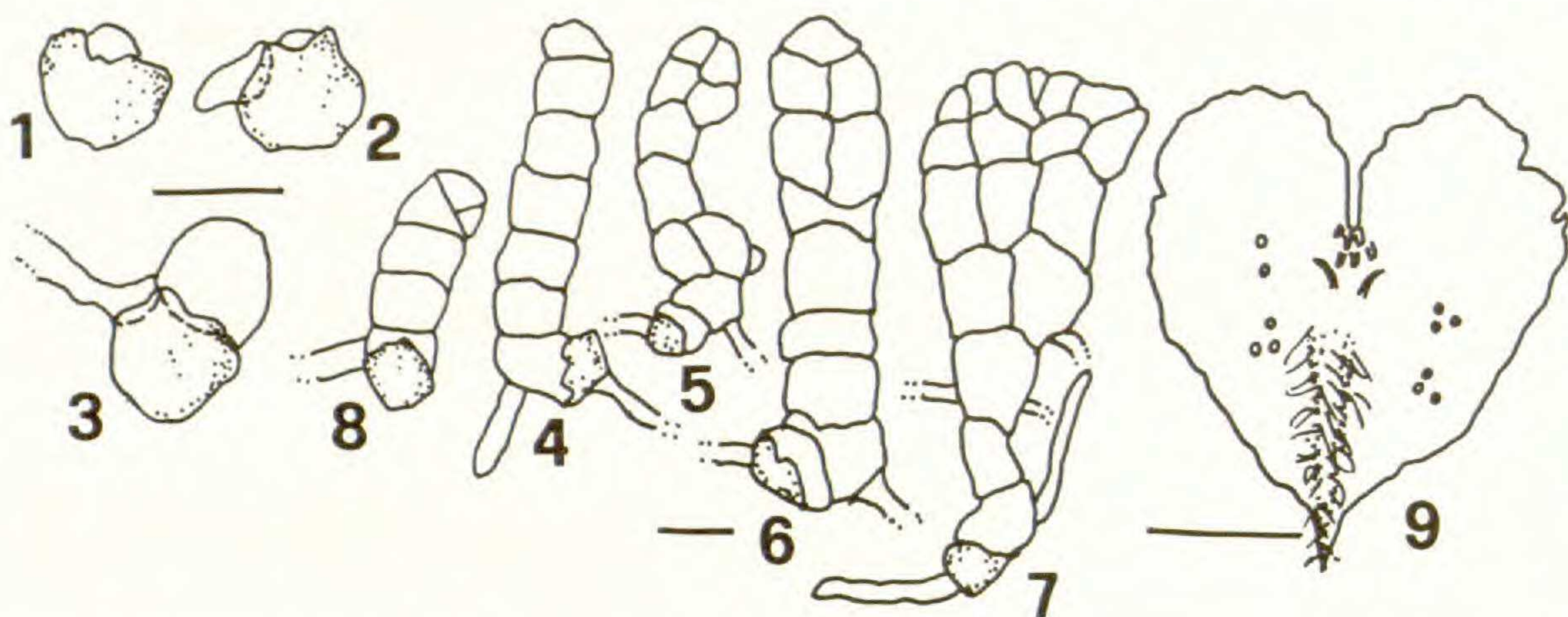
Microscopes (Lecica, Wild M8; Leitz, Dialux 20) were used to make morphological observations of gametophytes and young sporophytes. Pictures were made with the aid of a drawing tube or photomicrography. Gametangia were also observed with a scanning electron microscope. Gametophytes were dry mounted on double-stick mending tape, coated with gold, and observed with a Hitachi S2400 Scanning Electron Microscope.

RESULTS

The number of sporangia per sorus averaged 50.7 ± 13.1 s.d. and ranged from 19 to 78. Each sporangium contained 64 spores.

On agar medium, germination of spores stored at 4°C for one or two years began about 5 days after sowing, whereas germination of fresh spores did not begin until seven days after sowing. The viability of spores maintained for one or two years in cold storage was not significantly less than that of fresh spores. Two weeks after sowing, over 95% of fresh and stored spores had germinated (Table 2).

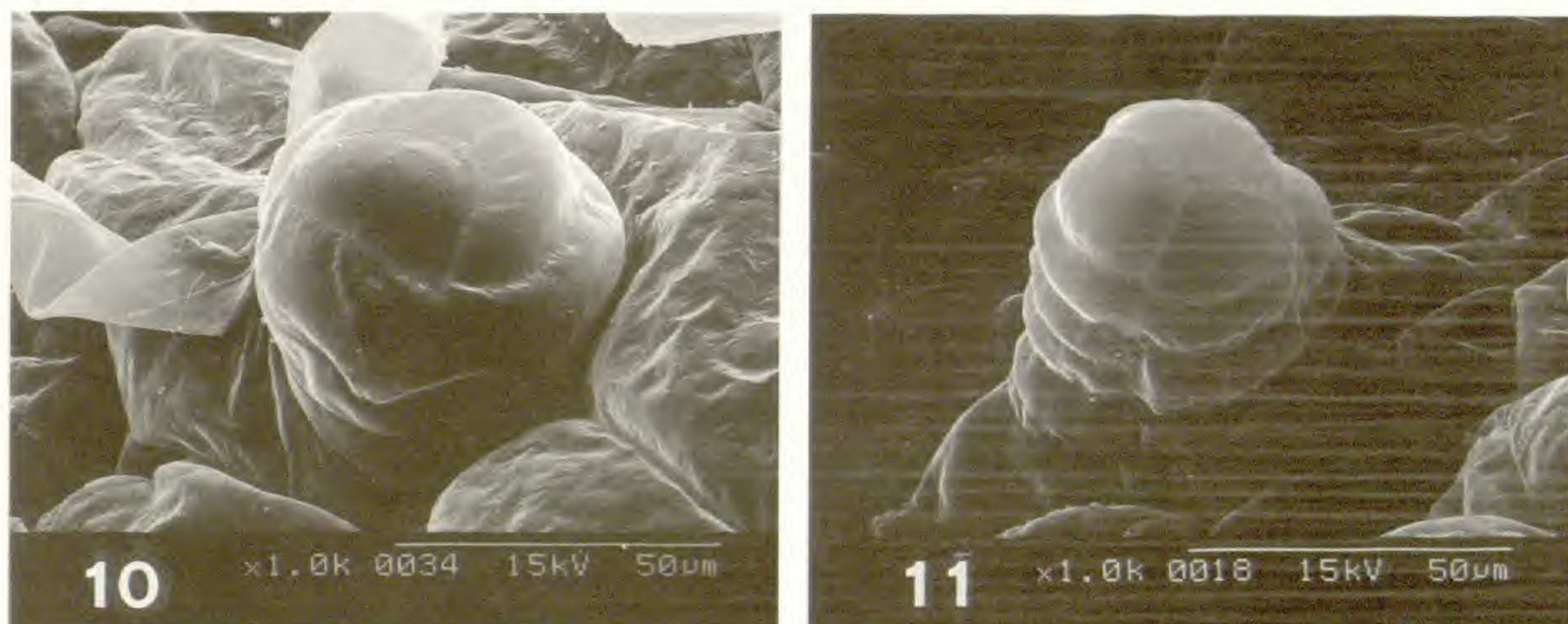
Spores of *S. lepifera* are tetrahedral. When spores germinate, the spore wall ruptures at the triradiate ridge (Fig. 1). The first cell division was parallel to the spore axis and formed the first rhizoid (Fig. 2), which elongated rapidly and did not contain chloroplasts. The second division was perpendicular to



FIGS. 1–9. Morphology of different stages of *S. lepifera* gametophytes. Figs. 1–3. Spore germination. Figs. 4–6. Filamentous stages. Fig. 7. A spatulate gametophyte. Fig. 8. A filament with a wedge-shaped apical cell. Fig. 9. A heart-shaped gametophyte. Bar = 50 μ m for Figs. 1–8; 1 mm for Fig. 9.

the first division (Fig. 3) and, by a series of transverse divisions, initiated a uniseriate filament that was usually 4–8 cells long (Figs. 4–8). About 4 days after spore germination, the apical, and sometimes subapical, cells divided parallel to the long axis of the filament (Fig. 5). However, in a few gametophytes the apical cell of the filament became sluggish and did not divide (Fig. 6). This apical cell was pushed to the subapical margin by intercalary divisions. Next, a spatulate plate formed. When gametophytes were about 5 cells wide, a wedge-shaped meristematic cell formed in the apical region (Fig. 7). At the filament stage, some gametophytes produced an apical meristematic cell (Fig. 8) that underwent repeated oblique divisions until it was replaced by a pluricellular meristem, the cells of which divided actively, producing a notched apex. About 5 weeks after spores were sown, a midrib formed behind the meristem in the median part, and a symmetrical, heart-shaped gametophyte formed (Fig. 9). The wings were one cell thick and were usually flat, but some curved upward at the margin. Rhizoids were restricted mostly to the ventral surface of the midrib. They were transparent but became light brown to brown with age.

Gametophytes initiated antheridium production in the spatulate stages (<0.5 mm wide). In heart shaped gametophytes, antheridia usually appeared on the ventral surface of the wings (Fig. 9), with a few forming on the dorsal surface. If the apical notch had not formed, antheridia sometimes formed near the apical region (Fig. 17), or on the margin, especially on filamentous proliferations (Fig. 20). Mature antheridia were about 50 μ m wide and 40 μ m high (Fig. 10). The antheridium wall was composed of five cells: a basal cell, a lower ring cell, an upper ring cell, a crescent-shaped cell, and an elliptical opercular cell. Upon dehiscence, the opercular cell was thrown off, and sperm were released. Gametophytes did not produce archegonia until 7 weeks after spore sowing (Table 3). Archegonia were restricted to the midrib behind the notch on the ventral surface, and no female only gametophytes were observed (Table 3).



FIGS. 10–11. SEM of *S. lepifera* gametangia. Fig. 10. An antheridium. Fig. 11. An archegonium.

Mature archegonia were about 40 μm wide and 50 μm high (Fig. 11), and the neck was 4 to 5 cells long. Antheridia continued to form after archegonia were initiated. Male gametophytes were usually less than 3 mm wide, whereas hermaphroditic gametophytes were usually greater than 3 mm wide. By 11 weeks after spore sowing, all gametophytes bore antheridia, that is they were male or hermaphroditic (Table 3).

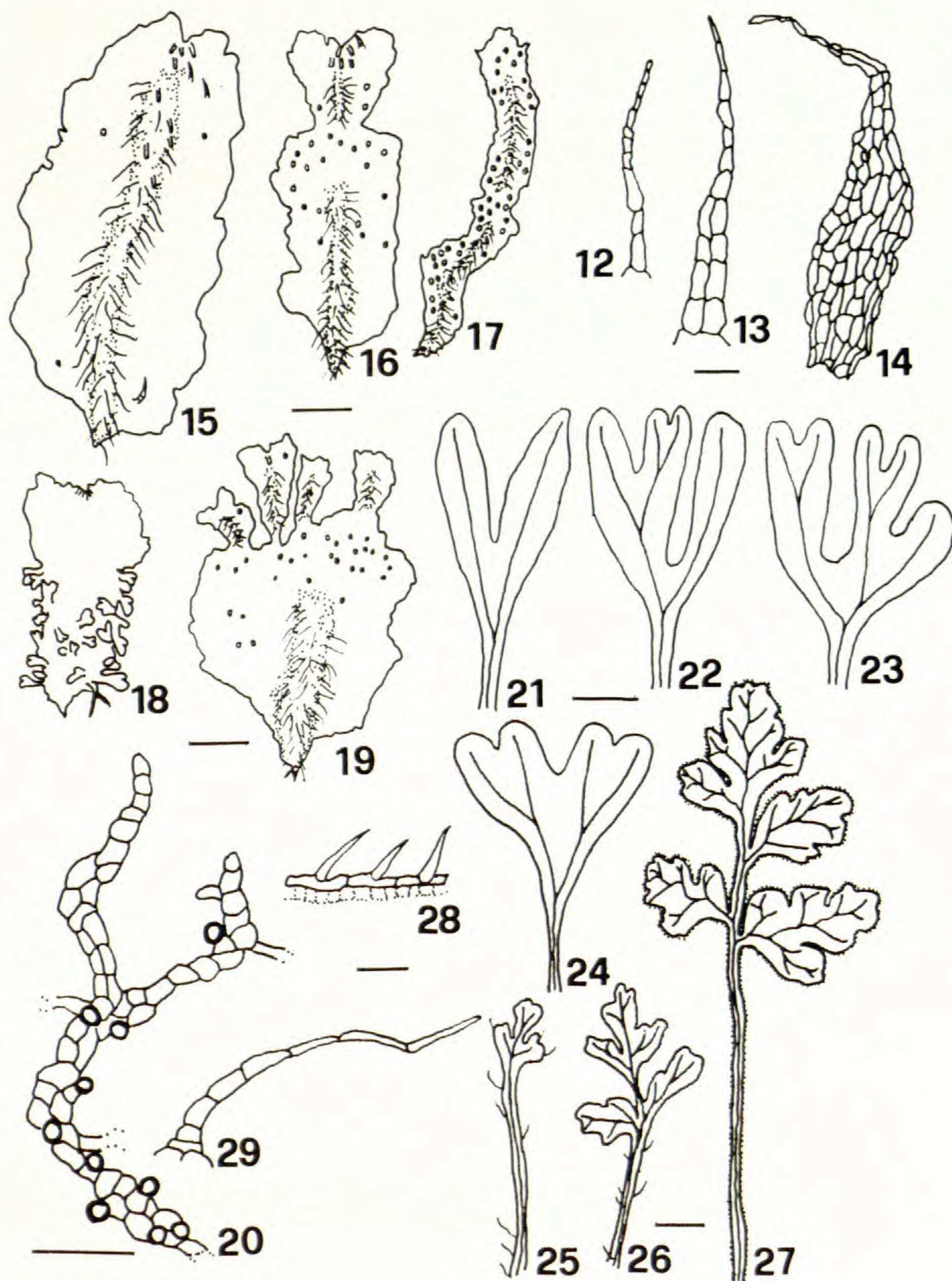
Trichomes occurred on both the dorsal and ventral surfaces of the gametophyte midrib. Usually, they were mixed with the archegonia on the cushion, especially near the apical notch of the gametophyte. Trichomes did not appear until the cushion and archegonia formed. The size of the pluricellular hairs varied greatly. Those on young gametophytes were uniseriate or, occasionally, bi- or triseriate (Figs. 12, 13), and usually were composed of less than 20 cells. Hairs began as uniseriate structures that grew by intercalary division into larger, multiseriate structures with age. On older gametophytes (e.g., 8 months old), the hairs could grow to 8 cells wide and be comprised of more than 50 cells in total, which were with a uniseriate, needle-like tip (Fig. 14).

Some gametophytes of *S. lepifera* elongated and became elliptical as they aged (e.g., 15 weeks old) (Figs. 15–17). Older gametophytes often became pale, or even necrotic, at their posterior end but kept growing at their anterior end. Some were slender, with many antheridia, but without archegonia or an apparent apical meristem. Other elongate gametophytes formed discrete cushions (Figs. 16, 17).

Some gametophytes began to produce vegetative proliferations when they were 13 weeks old. Daughter gametophytes formed from one or a few cells on the dorsal or ventral surfaces or on the margin (Fig. 18). Each of the daughter gametophytes developed a pluricellular meristem and gametangia and eventually produced large clones. Some gametophytes formed many filamentous proliferations, one to several cells wide (Fig. 20), that produced only antheridia. Occasionally, the anterior wings of gametophytes elongated and proliferated (Fig. 19). As proliferations grew, their posterior died, separating them from the parent gametophyte.

TABLE 3. Sexual status (%) and size (mm) of *S. lepifera* gametophytes of different ages grown in multispore cultures on peat media. A = asexual, M = male, F = female, H = hermaphroditic.

Width	5 wk						7 wk						9 wk						11 wk						13 wk						15 wk					
	A	M	F	H	A	M	F	H	A	M	F	H	A	M	F	H	A	M	F	H	A	M	F	H	A	M	F	H								
0.5	32	38	—	—	5	30	—	—	—	—	—	—	—	23	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
1	1	24	—	—	1	36	—	—	—	—	—	—	1	8	—	—	—	—	21	—	—	—	—	—	—	5	—	—								
2	—	5	—	—	—	25	—	—	—	—	—	—	—	40	—	—	5	—	44	—	—	—	—	—	—	38	—	—								
3	—	—	—	—	—	1	—	2	—	—	—	9	—	19	—	—	16	—	14	—	—	—	—	—	—	13	—	—								
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—								
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
Total	33	67	—	—	6	92	—	2	1	90	—	9	—	—	—	21	—	63	—	—	—	—	—	—	—	—	40	—								



FIGS. 12-29. Morphology of gametophytes and juvenile sporophytes of *S. lepifera*. Figs. 12-14. Multicellular hairs of gametophytes. Figs. 15-17. Elongate gametophytes. Figs. 18, 19. Gametophytes with proliferations. Fig. 20. Filamentous proliferation with antheridia. Figs. 21-24. Midribless first few fronds. Figs. 25-27. Midribbed fronds. Fig. 28. Unicellular hairs of a young sporophyte. Fig. 29. Multicellular hair of a young sporophyte. Bar = 100 μ m for Figs. 12-14, 28, 29; 0.2 mm for Fig. 20; 1 mm for Figs. 15-17, 21-27; 2 mm for Figs. 18, 19.

If water sufficient for fertilization was supplied, young sporophytes appeared in peat cultures about 12 weeks after spore sowing. The gametophyte usually senesced and disappeared after the sporophyte formed. However, some gametophytes with a young sporophyte formed proliferations that produced additional sporophytes. Laminae of the first few fronds of young sporophytes branched dichotomously (Figs. 21–24). Subsequent juvenile fronds were pinnately divided (Figs. 25–27). Except for the first few fronds, unicellular hairs and scales were evenly distributed on the frond margin (Figs. 25–29).

DISCUSSION

Sen (1964) noted that the diagnostic characters for the sporangia of most genera in the Cyathaceae are very similar. In the Cyathaceae, the number of spores in each sporangium is 64 or 16. The sporangia of *Lophosoria*, *Sphaeropteris*, *Trichipteris*, *Cyathea*, *Cnemidaria* and, probably, *Metaxya* contain 64 spores, whereas those of most species of *Alsophila* and all species of *Nephelea* have 16 spores (Gastony, 1974). In this study, 64 spores were found in *S. lepifera* sporangia, the same number found in the sporangia of the majority of more highly evolved leptosporangiate ferns (Holttum and Sen, 1961; Gastony, 1974).

Page (1979) reported that the spores of some Cyatheaceae lost their viability after a few weeks, but did not describe the conditions under which they were stored. Germination of *Cyathea delgadi* spores was less than 20% after 2 years of dry storage at -12°C (Simabukuro *et al.*, 1998). However, the high germination rates of *S. lepifera* spores, even after 2 years of cold storage, suggest that the viability of Cyatheaceae spores varies from species to species and may be dependent on the storage conditions.

After *S. lepifera* spores germinated, the filament grew along the polar axis, while the first rhizoid elongated along the equatorial plane. This pattern of spore germination has been classified as “*Cyathea*-type” and is considered typical of ferns in the Cyatheaceae (Nayar and Kaur, 1971).

Using the definitions of Nayar and Kaur (1971), *S. lepifera* gametophyte development was mostly of the *Drynaria*-type, but with some *Adiantum*-type features. Our results are somewhat different from those of Nayar and Kaur (1971), who described *S. lepifera* gametophyte development as *Adiantum*-type or nearly *Drynaria*-type.

Although most *S. lepifera* gametophytes were typical heart-shaped, some became more elongated. Elongate gametophytes were paler and more slender than heart-shaped gametophytes, and some remained in the antheridial stage for 8 months. Stokey (1930) reported that elongate gametophytes of *Cyathea arborea* were very much pronounced and produced only antheridia when they grew in weak light. If low light intensity causes *S. lepifera* gametophytes to elongate, the slender, elongate gametophytes observed in this study may have developed after being overgrown by other gametophytes. This simulates partial burial, which may occur in nature. Although elongate gametophytes are in-

capable of supporting sporophytes, they may play a significant role in providing sperm and thus promoting cross-fertilization.

In the Cyatheaceae, the antheridial wall is usually composed of 5 cells, but some variation occurs (Stokey, 1930; Atkinson and Stokey, 1964). The wall of each antheridium of *S. lepifera* in this study was always comprised of 5 cells, including a basal cell, 2 ring cells, a crescent-shaped cell and an elliptical opercular cell that was usually shed at antheridial dehiscence. The archegonium neck is straight, more or less, as has been reported for other Cyatheaceae and some less advanced ferns (Stokey, 1930; Atkinson and Stokey, 1964).

The peculiar, multicellular hairs on the archegonial cushion of the gametophyte are characteristic of Cyatheaoid gametophytes (Stokey, 1930). Outside the Cyatheaceae, similar multicellular hairs are found only on gametophytes in the Loxsomaceae (Stokey, 1960; Atkinson and Stokey, 1964). Homology of the multicellular hairs on gametophytes in the Cyatheaceae and Loxsomaceae is supported by molecule-based cladistic analysis (Pryer *et al.*, personal communication).

Gametophytes of *S. lepifera* can produce large clones and enhance their longevity by vegetative growth, as has been described for some other species in the Cyatheaceae and many other fern taxa (Stokey, 1930; Atkinson and Stokey, 1964; Chiou and Farrar, 1997a). The type of vegetative regeneration present in *S. lepifera*, in which new gametophytes formed on the surface or margins of established gametophytes, also was observed in *Alsophila excelsa* by Atkinson and Stokey (1964). They suggested that this type of vegetative regeneration usually occurred on old or injured gametophytes. However, many clones of *S. lepifera* formed from gametophytes that were green and very healthy. Although each new gametophyte could produce male and female gametangia, few produced sporophytes while still attached to the parent gametophyte if the parent plant had produced a sporophyte. Perhaps, the developing embryo of the parent gametophyte inhibited the fertilization of eggs of the daughter gametophytes, or inhibited zygote development in fertilized eggs. The mechanism for this inhibition needs further investigation.

The sexual expression of *S. lepifera* is related to gametophyte size and development stages. Most asexual gametophytes were less than 0.5 mm wide, while male only gametophytes were 0.5–3.0 mm wide, and hermaphroditic gametophytes were usually more than 3.0 mm in width. Initial antheridium formation was followed by the attainment of a prolonged hermaphroditic phase during which antheridia continued to form. However, in a previous study of the gametangial sequence of *S. lepifera* (= *Cyathea lepifera*), antheridium formation ceased once plants became hermaphroditic (Masuyama, 1975b). Gametophyte sexual expression may be affected by the culture medium (Masuyama, 1975a). Masuyama's (1975b) observations were on gametophytes grown on agar medium, whereas gametophytes in this study were grown on peat medium.

A gametangial sequence from male to hermaphroditic may favor intragametophytic selfing (Klekowski, 1969; Masuyama, 1975a). However, this type of mating system is not consistent with another study of *S. lepifera* in Taiwan.

Based on isozyme data, Chen (1995) suggested that the mating system of *S. lepifera* tended toward intergametophytic crossing. Similarly, three other tree ferns, *Alsophila firma* (Cyatheaceae), *Cyathea stipularis* (Cyatheaceae) and *Lophosoria quadripinnata* (Lophosoriaceae) are outcrossing (Soltis *et al.*, 1991). It has been suggested that inbreeding of bisexual gametophytes may be limited by high genetic load (Klekowski, 1969, 1973; Masuyama, 1979; Peck *et al.*, 1990; Hooper and Haufler, 1997). Thus, if bisexual gametophytes are common in natural populations of *S. lepifera*, genetic load may be the most important factor favoring outcrossing in *S. lepifera*.

Young sporophytes were produced when sufficient water was supplied to sexually mature plants. Gametophytes would not bear sporophytes when water was not added to cultures, although they had mature gametangia. In addition, young sporophytes were always born on the archegonia, suggesting that *S. lepifera* sporophytes likely resulted from fertilization. The 64-spore sporangium also indicates that it is not an apogamous species.

In *S. lepifera*, the blades of juvenile leaves are dichotomously branched, whereas those of succeeding juvenile fronds are pinnately dissected. This is the most common type of frond ontogeny in leptosporangiate ferns (Wagner, 1952).

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